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IN FOCUS

Coronary artery calcification score and carotid intima–media thickness in patients with hemophilia

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Summary. *Background/objectives:* The traditional view that patients with hemophilia are protected against cardiovascular disease is under debate. The aim of the present study was to evaluate the presence and extent of atherosclerosis by coronary artery calcification score (CACS) and carotid intima media thickness (IMT) in patients with hemophilia, and to evaluate their cardiovascular risk profile. *Methods:* Sixty-nine patients (51 with hemophilia A; 18 with hemophilia B) were studied [median age: 52 years (interquartile range [IQR] 43–64)]. Cardiovascular risk factors and prior major adverse cardiovascular events (MACEs) were recorded. CACS was derived from electron-beam or dual-source computed tomography, and carotid IMT was assessed by ultrasound measurements and compared with age-specific reference values. *Results:* The median CACS in all patients was 35 (IQR 0–110) and the geometric mean IMT was 0.80 mm (95% confidence interval [CI] 0.76–0.84); neither was different from the reference values. Patients with a previous MACE ($n = 9$) had significantly higher CACS and IMT than patients without a previous MACE: CACS median 1013 (IQR 530–1306) vs. 0 (IQR 0–67), and IMT geometric mean 1.09 mm (95% CI 0.95–1.26) vs. 0.76 mm (95% CI 0.73–0.79), both $P < 0.001$. A higher calculated 10-year cardiovascular risk was related to higher IMT and CACS. *Conclusion:* Patients with hemophilia are not protected against the development of atherosclerosis as measured by CACS and IMT. The extent of atherosclerosis is related to the traditional cardiovascular risk factors. This suggests that traditional cardiovascular risk factors should be monitored and treated in patients with hemophilia.

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Introduction

The traditional view that patients with hemophilia are protected against cardiovascular disease is based on several European and US studies that found lower mortality rates for ischemic heart disease in persons with hemophilia than in the general population, carried out in the period between 1968 and 1993 [1–3]. It was suggested that the hypocoagulable state and a lower prevalence of cardiovascular risk factors could account for this. However, recent reports challenge this view. The presence of coronary artery disease and the lifetime prevalence of cardiovascular events in patients with hemophilia were found to be similar to those in the general population [4–6]. Furthermore, in patients with hemophilia and cardiovascular disease, the presence of coronary disease and cardiovascular morbidity and mortality were associated with the same traditional risk factors as in the general population. Cardiovascular disease in patients with hemophilia has attracted more interest in recent years, because decreased mortality from bleedings and viral infections has resulted in a longer life-expectancy and consequently longer exposure to cardiovascular risk factors [7–9].

The sequence of events from the earliest atherosclerotic changes in the arterial wall to a clinical cardiovascular event is a gradual process, starting with a fatty streak in the vessel wall, followed by inflammation, calcification, plaque rupture, and thrombosis [10]. Consequently, a possible lower prevalence of

cardiovascular events in hemophilia patients could result from an alteration of any part of this sequence. Theoretically, diminished development of atherosclerosis, diminished plaque vulnerability and a lower tendency to coagulate could contribute to a decreased risk of a cardiovascular event in a patient with hemophilia. The present study focused on the presence and degree of atherosclerosis in this process.

Earlier studies on atherosclerosis using intima–media thickness (IMT) measurements of carotid and femoral arteries in patients with coagulopathy gave discordant results. A study of 76 patients with hemophilia or von Willebrand's disease showed no clinically relevant differences in IMT between patients and healthy controls [11]. A second study that could not find a difference in IMT did not report the age of the control group. This is unfortunate, as age is still the most important determinant of IMT [12]. Two studies in 76 and 40 patients with hemophilia A, respectively, reported fewer carotid plaques, a smaller degree of carotid stenosis, and a lower number of plaques in the aorta and leg arteries [13,14]. Also, a case–control study found a significantly lower IMT in 50 patients with hemophilia than in age-matched and sex-matched controls, whereas their cardiovascular risk factors were comparable [15].

The coronary artery calcification score (CACS) is derived from new-generation computed tomography (CT) scans of the heart, and can be used to detect and quantify subclinical atherosclerosis. Like IMT, CACS can identify atherosclerosis even at a subclinical level. Furthermore, CACS is currently the only non-invasive measurement of atherosclerosis at the heart. Both methods are highly predictive for cardiovascular events, even in the general population and patients at low to intermediate cardiovascular risk [16–20].

There are no reports on CACS as measure of subclinical atherosclerosis in patients with hemophilia. The aim of our study was to evaluate the presence and extent of atherosclerotic lesions by CACS and IMT, and to assess the cardiovascular risk profile in patients with hemophilia.

Materials and methods

Study design

A cross-sectional study was performed in a cohort of unselected patients with hemophilia A or B registered with the Hemophilia Treatment Center of the University Medical Center Groningen from 2006 to 2009.

Participants

Eligible patients were male, at least 30 years of age, and had hemophilia A or B. Patients were invited to visit the outpatient clinic or were included during their regular visit.

Cardiovascular risk factors

All traditional cardiovascular risk factors were assessed. Hypertension was defined as either a systolic blood pressure of > 140 mmHg or a diastolic blood pressure of > 90 mmHg, or current use of blood pressure-lowering medication [21]. Overweight was defined as a body mass index of > 25 kg m^{-2}. Smoking status (current, past, or never), family history of premature cardiovascular disease (cardiovascular event in first-degree relatives, male aged < 55 years and female aged < 65 years) and use of blood glucose-lowering drugs were obtained with a questionnaire. The 10-year mortality risk for cardiovascular disease was calculated with the Systemic Coronary Risk Evaluation (SCORE) adjusted for the Dutch population, which is used for risk assessment in a primary prevention setting [22]. The UK Prospective Diabetes Study risk engine was used for patients with diabetes mellitus [23]. All patients with a prior major adverse cardiovascular event (MACE) were classified as having a high (> 10%) 10-year risk for cardiovascular mortality. MACE was defined as acute coronary syndrome (ACS), percutaneous coronary intervention (PCI), coronary artery bypass graft (CABG), peripheral arterial occlusive disease (PAOD), abdominal aortic aneurysm (AAA), peripheral revascularization interventions, stroke, and carotid revascularization.

Laboratory testing

Total cholesterol, triglycerides, LDL cholesterol and HDL cholesterol were assessed in a fasting state (Modular; Roche Diagnostics, Almere, The Netherlands).

CT for coronary calcium scoring

Scanning was performed on an electron-beam CT scanner (C-150 EBT; GE-Imatron, South San Francisco, CA, USA) for the first 44 patients, or on a dual-source CT scanner (SOMATOM Definition; Siemens Healthcare, Forchheim, Germany) for the remainder of the patients. Before the subjects were scanned, they practiced breath-holding. The scan range was from the level of the root of the aorta through the heart. Images were acquired at 80% of the cardiac cycle (at 70% for dual-source CT), with electrocardiogram triggering, during a single breath-hold. Quantification of coronary calcification was performed with dedicated software (in the case of electron-beam CT, AccuImage Diagnostics Corporation, South San Francisco, CA, USA; in the case of dual-source CT, Syngo, Siemens Healthcare). Trained scan readers were blinded to the clinical data of the participants. A calcification was defined as a minimum of two adjacent pixels (area = 0.52 mm^2) with a density over 130 Hounsfield Units (HU), based on the method in a population-based study [20]. A calcium score for the coronary arterial tree was calculated according to Agatston's method [24]. In this scoring method, the area (in square millimeters) of individual calcified lesions is multiplied by a factor based on the maximum density of the lesion. This factor ranges from 1 to 4 in the following manner: 1, 130–199 HU; 2, 200–299 HU; 3, 300–399 HU; and 4, ≥ 400 HU. The total calcium score was obtained by adding up the scores for all individual lesions. Electron-beam CT was replaced by

dual-source CT during the study period. The resulting CACS was comparable for the two scanning methods, as shown in a previous validation study [25]. CACS was compared with the reference values of the white ethnic subgroup ($n = 2619$) from the Multi-Ethnic Study of Atherosclerosis (MESA) cohort of patients free of cardiovascular disease [26]. In this study, each standard deviation (SD) increase in CACS was related to a 2.1-fold increased risk for future cardiovascular disease and a 2.5-fold increased risk for coronary heart disease in the next 5.3 years, corrected for traditional cardiovascular risk factors [26]. CACS was considered to be increased if the value was higher than the age-specific and sex-specific geometric mean + 1 SD according to the reference values. CACS was also classified into four different categories – 0–100, 101–400, 401–1000, and above 1000 – according to the categorization in the population-based Rotterdam Study [20]. These categories of increasing calcium scores are associated with increasing risk of cardiovascular events [20,27,28].

Carotid ultrasound imaging

We measured the carotid IMT as described previously [29]. High-resolution B-mode ultrasonography (Acuson 128XP10; Acuson Corporation, Mountainview, CA, USA) with a 7-MHz linear array transducer was used with the patient in a supine position. For both carotid arteries, the far wall segments of the common carotid artery and internal carotid artery were imaged from a fixed lateral transducer position. The sonographers were unaware of the risk factors of the studied persons. The measurements were analyzed online. An IMT that was thicker than the age-specific 80th percentile was considered to be abnormal [30]. As IMT measurement is not as well standardized worldwide as CACS, and regional differences have been reported [31], local reference values were used from healthy individuals.

Statistical analysis

As the IMT data are normalized by log transformation, the geometric means and 95% confidence intervals (CIs) ($\pm$ 2 $\times$ standard error of the mean) are presented. CACS data were processed after natural log of (CACS + 1) as in the MESA study [26]. Data analyses were performed by using SPSS version 16 (SPSS, Chicago, IL, USA). The chi-squared test (Fisher's exact test) was used for categorical data, the Mann–Whitney test for non-parametric data, and Student's *t*-test for normally distributed data. For comparisons across multiple groups, ANOVA and the Kruskal–Wallis test were used where appropriate.

Results

Participants and risk factors for cardiovascular disease

Patient characteristics are described in Table 1. One hundred and thirty-six male hemophilic patients were eligible, 69 of whom agreed to participate (51%). Forty-two did not respond to the invitation. Thirteen patients were irretrievable. Twelve patients declined to participate. Participants and non-participants did not differ in type or severity of hemophilia. Of the participants, 51 (74%) had hemophilia A and 18 (26%) had hemophilia B. Hemophilia was severe (factor VIII or FIX < 1%) in 27 patients, moderate (FVIII or FIX 1–5%) in eight, and mild (FVIII or FIX > 5%) in 34. Twenty-four were receiving prophylactic treatment. The median age was 52 years (interquartile range [IQR] 43–64). The cardiovascular risk profile is shown for the complete cohort and for the patients with and without a previous MACE (Table 1). As stated earlier, patients with a previous MACE are, by definition, at high cardiovascular risk. In contrast, the patients without a previous MACE had a low estimated 10-year risk for cardiovascular death: medians of 7% for the patients with diabetes and of 2% for the patients without diabetes.

Previous cardiovascular events

Nine patients (13%) had already experienced a MACE, including two with ACS and one with AAA. One was diagnosed with PAOD and underwent CABG surgery. Two had undergone CABG, one PCI, one PCI and CABG, and one PCI and left-sided and right-sided carotid desobstruction. Both ACS events occurred during clotting factor replacement for elective surgery. Patients with a previous MACE were older, and more often had hypertension, and were treated with blood pressure-lowering and cholesterol-lowering drugs (Table 1).

Coronary calcification

Figure 1A shows the CACS distribution and the age-dependent reference values. In three patients, CACS was not performed; two of them had a history of coronary disease, and one had peripheral occlusive arterial disease. CACS in all hemophilic patients (median 35, IQR 0–110) did not differ from the reference values. Nine (14%) patients had an increased CACS. Patients without a previous MACE had a median CACS of 0 (IQR 0–67), with the score being < 100 in 81% of patients (Table 2). In patients with a previous MACE, the median CACS was significantly higher (1013, IQR 530–1306, $P = 0.03$), with more patients being in a higher CACS class ($P < 0.001$). The median CACS was 13 in patients with severe hemophilia (IQR 0–251), 4 in those with moderate hemophilia (IQR 0–42), and 0 in those with mild hemophilia (IQR 0–99) ($P = 0.57$). No differences in CACS were seen when hemophilia A and B patients were compared: the median CACS was 8 in those with hemophilia A (IQR 0–123) and 0 in those with hemophilia B (IQR 0–101) ($P = 0.57$). The percentage of patients with a higher CACS was positively associated with an increasing SCORE risk, as shown in Fig. 2A ($P < 0.001$). The patients with diabetes mellitus are shown separately, as the SCORE risk calculation is not validated for diabetic patients.

Table 1 Patient characteristics and cardiovascular risk factors

	All patients (n = 69)	Patients without a MACE (n = 60)	Patients with a MACE (n = 9)	P-value
Patient characteristics				
Median age in years (IQR)	52 (43–64)	51 (42–62)	69 (61–74)	0.03
Type of hemophilia, no. (%)				
Hemophilia A	51 (74)	45 (75)	6 (67)	0.60
Hemophilia B	18 (26)	15 (25)	3 (33)	
Severity, no. (%)				
Severe	27 (39)	23 (38)	4 (44)	0.48
Moderate	8 (12)	7 (12)	1 (11)	
Mild	34 (49)	30 (50)	4 (44)	
HIV infection, no.	0	0	0	NA
Hepatitis C infection, no. (%)	41 (62)	37 (64)	4 (44)	0.45
Cardiovascular risk factors				
Overweight, no. (%)	36 (53)	31 (53)	5 (56)	0.87
Positive family history of premature cardiovascular events, no. (%)	13 (19)	9 (16)	4 (44)	0.06
Smoking, no. (%)				
Current	21 (31)	19 (33)	2 (22)	0.80
Former	29 (43)	25 (43)	4 (45)	
Never	17 (26)	14 (24)	3 (33)	
Blood pressure				
Median systolic blood pressure (mm Hg) (IQR)	130 (125–150)	130 (121–145)	160 (139–178)	0.007
Median diastolic blood pressure (mm Hg) (IQR)	80 (75–85)	80 (75–85)	80 (78–98)	0.12
Systolic hypertension, no. (%)	30 (44)	23 (38)	7 (78)	0.03
Diastolic hypertension, no. (%)	9 (13)	5 (8)	4 (44)	0.003
Use of antihypertensives, no. (%)	18 (27)	10 (17)	8 (89)	< 0.001
Median total cholesterol (mmol L^{-1}) (IQR)	4.9 (4.1–5.7)	4.9 (4.1–5.7)	4.6 (4.0–5.5)	0.80
Median triglycerides (mmol L^{-1}) (IQR)	1.4 (1.0–1.9)	1.4 (0.9–2.0)	1.5 (1.1–2.2)	0.47
Median HDL cholesterol (mmol L^{-1}) (IQR)	1.3 (1.1–1.5)	1.3 (1.1–1.5)	1.3 (1.0–1.5)	0.96
Median LDL cholesterol (mmol L^{-1}) (IQR)	3.2 (2.6–4.0)	3.2 (2.6–4.0)	2.9 (2.6–3.9)	0.25
Use of cholesterol-lowering drugs, no. (%)	8 (12)	3 (5)	5 (56)	< 0.001
Diabetes mellitus, no. (%)	7 (10%)	6 (10%)	1 (11%)	0.92

HIV, human immunodeficiency virus; IQR, interquartile range; MACE, major adverse cardiovascular event; NA, not applicable. All P-values are for comparison between patients with and without a previous MACE. Chi-squared Fisher's exact test for categorical data; Mann–Whitney test for continuous variables.

IMT

IMT was measured in 65 patients. Four patients cancelled their appointment for unknown reasons. These four patients did not have a previous MACE, and all had an evaluable CACS. Carotid IMT results and age-dependent reference values are shown in Fig. 1B. The mean carotid IMT in hemophilic patients was 0.80 mm (95% CI 0.76–0.84 mm) (Table 2), and did not differ from the reference values. The median carotid IMT was significantly higher in patients with a previous MACE than in patients without a previous MACE: 1.09 mm (95% CI 0.94–1.25 mm) vs. 0.76 mm (95% CI 0.73–0.79 mm) (P < 0.0010). For five patients, comparison with the reference value was not possible, because they were aged > 70 years. Eighteen of the 65 patients (28%) had an IMT above the 80th percentile of the age-dependent reference values, and were considered to be abnormal. The mean IMT was 0.74 mm in patients with severe hemophilia (95% CI 0.69–0.80 mm), 0.77 mm in those with moderate hemophilia (95% CI 0.64–0.94 mm), and 0.76 mm in those with mild hemophilia (95% CI 0.72–0.81 mm) (P = 0.81). No differences in IMT were seen when hemophilia A and B patients were compared: the mean IMT was 0.75 mm in hemophilia A patients (95% CI 0.71–0.80 mm) and 0.76 mm in hemophilia B patients (95% CI 0.69–0.84 mm) (P = 0.78). There was a clear relationship between the SCORE risk and IMT (Fig. 2B). With ANOVA, differences in carotid IMT were significant for the different risk groups (P < 0.001).

Discussion

This study evaluated the presence of atherosclerosis in patients with hemophilia as measured by CACS and IMT. The extent of atherosclerosis was in accordance with the normal age-dependent reference values for both measures, and was independent of hemophilia type or severity. Therefore, these data do not confirm the alleged protection given by hemophilia against atherosclerosis [13–15]. Moreover, hemophilic patients with a previous MACE had increased CACS and/or IMT. This suggests similar development of atherosclerosis as in patients

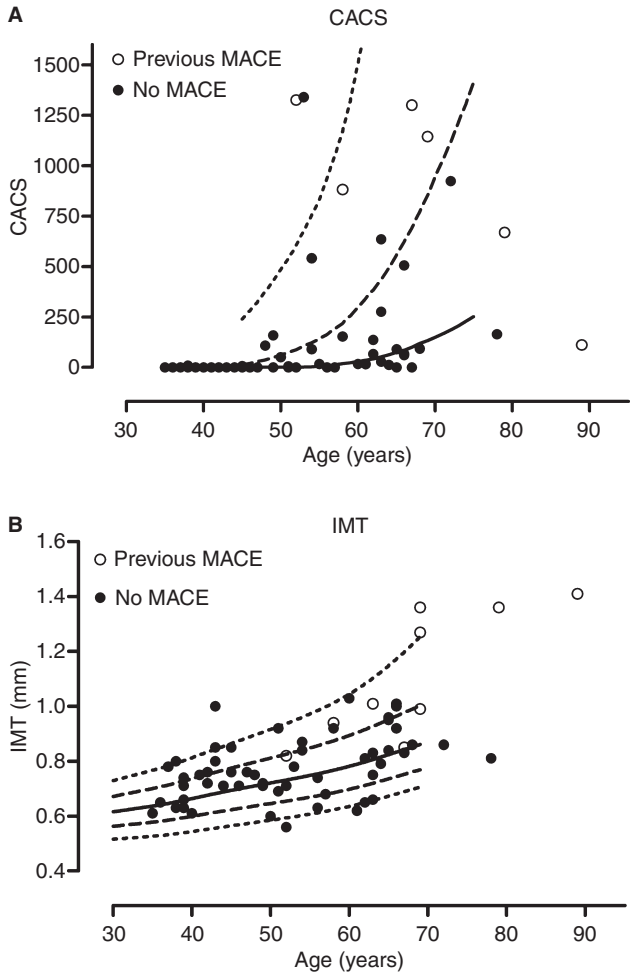

Fig. 1. Measures of subclinical atherosclerosis in patients with haemophilia. (A) Coronary artery calcification score (CACS) in patients with hemophilia with and without a previous major adverse cardiovascular event (MACE). Straight line: mean reference value of CACS. Dotted line: mean + 1 standard deviation (SD) reference value of CACS. Fine dotted line: mean + 2 SD reference value of CACS. (According to the Multi-Ethnic Study of Atherosclerosis data.) (B) Intima–media thickness (IMT) in patients with hemophilia with and without a previous major adverse cardiovascular event (MACE). Straight line: 50th percentile of local reference values. Dotted lines: 20th and 80th percentiles of local reference values. Fine dotted line: 5th and 95 percentiles of local reference values.

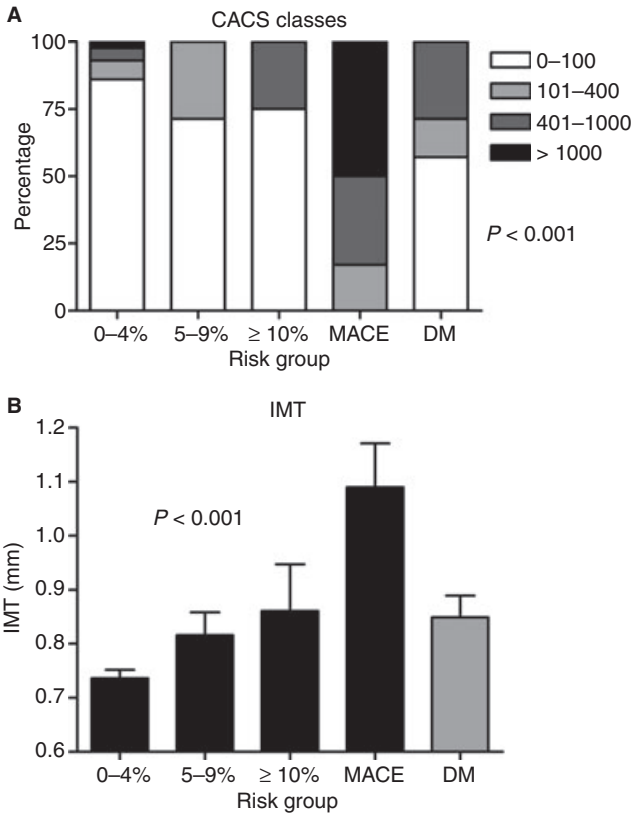

Fig. 2. Measures of subclinical atherosclerosis according to different cardiovascular risk groups. Coronary artery calcification score (CACS) classes (A) and intima–media thickness IMT (B) of the groups with different estimated risk according to the Systemic Coronary Risk Evaluation risk classification: the group after a previous major adverse cardiovascular event (MACE), and the group with type 2 diabetes mellitus (DM). $P < 0.001$ for the differences in CACS class for the different risk groups by the chi-squared test. $P < 0.001$ for the differences in carotid IMT for the different risk groups by ANOVA.

without hemophilia, where increased CACS and/or IMT is found in populations at high cardiovascular risk, such as after a MACE [16–19]. Furthermore, in patients without a previous MACE, CACS and IMT were positively correlated with the estimated cardiovascular risk class: the higher the risk class, the

Table 2 Coronary artery calcification score (CACS) and intima–media thickness (IMT)

	All patients (n = 69)	Patients without a MACE (n = 60)	Patients with a MACE (n = 9)	P value
CACS, median (IQR)	35 (0–110)	0 (0–67)	1013 (530–1306)	0.03
CACS in classes, no. (% of evaluable patients)				
0–100	49 (74)	49 (81)	0	< 0.001
101–400	7 (11)	6 (10)	1 (17)	
401–1000	6 (9)	4 (7)	2 (33)	
> 1000	4 (6)	1 (2)	3 (50)	
Carotid IMT (mm), geometric mean (95% CI)	0.80 (0.76–0.84)	0.76 (0.73–0.79)	1.09 (0.95–1.26)	0.003

CI, confidence interval; IQR, interquartile range; MACE, major adverse cardiovascular event. CACS was available in 66 patients; in three patients with a previous MACE, CACS was not assessed. All P-values are for comparison between patients with and without a previous MACE, corrected for age. Mann–Whitney test for CACS median; chi-squared Fisher's exact test for CACS classes; Student's t-test after log transformation for IMT.

higher CACS and IMT. CACS and IMT were not related to the severity of hemophilia. Together, these data suggest that the development of atherosclerosis is dependent on the traditional risk factors rather than being influenced by hypocoagulability.

It is important to note that CACS and IMT both quantify only a part of the sequence of atherosclerotic changes in the arterial wall that can eventually lead to thrombosis and a clinical cardiovascular event. Therefore, this study does not answer the question of whether hemophilic patients are, indeed, relatively protected from cardiovascular events.

Our results are in line with two other studies that did not find lower IMT in patients with hemophilia [11,12]. The difference in findings as compared with three studies reporting lower IMT may be explained by several factors. In the first study by Bilora, a mixed group of patients with hemophilia and von Willebrand's disease was studied, but the exact number of hemophilic patients and their specific characteristics were not reported [13]. Furthermore, the prevalence of plaques and degree of stenosis were studied rather than the IMT value itself, the exact definition of plaque was unclear, and it is questionable whether the selected controls were really free from atherosclerotic risk factors, as plaques were observed in 42 of the 77 controls. Similarly, in the second Bilora study, a mixed group of patients with hemophilia and von Willebrand's disease was studied [14]. Another important difference from our study concerns the outcome measures, as they studied the aorta, the femoral artery, and the popliteal artery, whereas we studied the carotid arteries. Again, the definition of plaques was unclear, the specific characteristics of the hemophilic patients were not given, and the authors did not report on whether or not the presence of atherosclerotic plaques was related to the atherosclerotic risk factors. In the third Bilora study, the carotid arteries, the aorta and the brachial and femoral arteries were studied. However, there was an inconsistent description of their study group; they were referred to as patients with hemophilia in some places in the paper, and as carriers of hemophilia in other places [15]. Nevertheless, we cannot give a clear explanation for their different IMT findings. It is remarkable that they found higher IMT in the common carotid artery than at the site of the carotid bulb in the hemophilic patients. In contrast, within the control group, the absolute mean IMTs of the common carotid artery and the carotid bulb were the same. In our study, we found a higher IMT in the carotid bulb than in the common carotid artery in the hemophilic patients (data not shown), as is usually reported in the literature. Thus, a difference in scanning technique could contribute to the discrepancies.

Our study has some limitations. First, the percentage of non-responders was 49%. In the group of non-responders, patients without complications (including a previous MACE), who rarely visit the outpatient clinic, were overrepresented. This could have led to selection bias, although there was no difference in the severity or type of hemophilia between responders and non-responders. Second, the number of included patients ($n = 69$) did not allow us to perform subgroup analyses with sufficient power.

A strong aspect of our study is the use of CACS as a measure for atherosclerosis. Not only has CACS never been studied before in hemophilic patients, but the technique for measuring CACS is standardized worldwide, in contrast to the IMT technique, the absence of consensus for which makes it difficult to compare the results of different study groups. For example, there are large differences in ultrasound transducer position and angle, which artery segments are measured (common carotid, internal carotid, and/or carotid bulb), in the use of the far wall or near wall, in whether the mean or the maximum IMT is used, and in whether or not the results of different segments are averaged. As a consequence, each IMT study needs its own local reference values or a separate control group. For CACS, the MESA values are the worldwide reference values derived from a healthy cohort, from which we used the data of 2619 Caucasian control subjects [26].

In conclusion, patients with hemophilia are not protected against the development of atherosclerosis. Their extent of atherosclerosis is related to the calculated risk based on the traditional cardiovascular risk factors. These findings suggest that they should be monitored for traditional cardiovascular risk factors and receive counselling and preventive measures, like any other person at risk for cardiovascular events.

Addendum

M. Zwiers, J. D. Lefrandt, A. V. M. Brands-Nijenhuis, and K. Meijer: conceived the study idea; M. Zwiers, J. D. Lefrandt, D. J. Mulder, A. J. Smit, R. O. B. Gans, R. Vliegenthart, A. V. M. Brands-Nijenhuis, J. C. Kluin-Nelemans, and K. Meijer: contributed to the study design, and data abstraction and interpretation; M. Zwiers and J. D. Lefrandt: wrote the manuscript. All authors took part in its revision and approved the final version.

Acknowledgement

J. van der Meer, who passed away in January 2009, was involved in planning this study.

Disclosure of Conflict of Interests

K. Meijer received research support from Baxter and Bayer, and was member of an advisory board for CSL Behring (manufacturers of coagulation factor concentrates). This study was supported by an unrestricted grant from Bayer.

References

1 Aronson DL. Cause of death in hemophilia A patients in the United States from 1968 to 1979. *Am J Hematol* 1988; **27**: 7–12.

2 Koumbarelis E, Rosendaal FR, Gialeraki A, Karafoulidou A, Noteboom WM, Loizou C, Panayotopoulou C, Markakis C, Mandalaki T. Epidemiology of haemophilia in Greece: an overview. *Thromb Haemost* 1994; **72**: 808–13.

3 Rosendaal FR, Varekamp I, Smit C, Brocker-Vriends AH, van Dijck H, Vandenbroucke JP, Hermans J, Suurmeijer TP, Briet E. Mortality and causes of death in Dutch haemophiliacs, 1973–86. *Br J Haematol* 1989; **71**: 71–6.

4 Foley CJ, Nichols L, Jeong K, Moore CG, Ragni MV. Coronary atherosclerosis and cardiovascular mortality in hemophilia. *J Thromb Haemost* 2010; **8**: 208–11.

5 Ragni MV, Moore CG. Atherosclerotic heart disease: prevalence and risk factors in hospitalized men with haemophilia A. *Haemophilia* 2011; **17**: 867–71.

6 Sharathkumar AA, Soucie JM, Trawinski B, Greist A, Shapiro AD. Prevalence and risk factors of cardiovascular disease (CVD) events among patients with haemophilia: experience of a single haemophilia treatment centre in the United States (US). *Haemophilia* 2011; **17**: 597–604.

7 Darby SC, Kan SW, Spooner RJ, Giangrande PL, Hill FG, Hay CR, Lee CA, Ludlam CA, Williams M. Mortality rates, life expectancy, and causes of death in people with hemophilia A or B in the United Kingdom who were not infected with HIV. *Blood* 2007; **110**: 815–25.

8 Diamondstone LS, Aledort LM, Goedert JJ. Factors predictive of death among HIV-uninfected persons with haemophilia and other congenital coagulation disorders. *Haemophilia* 2002; **8**: 660–7.

9 Triemstra M, Rosendaal FR, Smit C, van der Ploeg HM, Briet E. Mortality in patients with hemophilia. Changes in a Dutch population from 1986 to 1992 and 1973 to 1986. *Ann Intern Med* 1995; **123**: 823–7.

10 Berliner JA, Navab M, Fogelman AM, Frank JS, Demer LL, Edwards PA, Watson AD, Lusis AJ. Atherosclerosis: basic mechanisms. Oxidation, inflammation, and genetics. *Circulation* 1995; **91**: 2488–96.

11 Sramek A, Reiber JH, Gerrits WB, Rosendaal FR. Decreased coagulability has no clinically relevant effect on atherogenesis: observations in individuals with a hereditary bleeding tendency. *Circulation* 2001; **104**: 762–7.

12 Sartori MT, Bilora F, Zanon E, Varvarikis C, Saggiorato G, Campagnolo E, Pagnan A, Cella G. Endothelial dysfunction in haemophilia patients. *Haemophilia* 2008; **14**: 1055–62.

13 Bilora F, Dei Rossi C, Girolami B, Casonato A, Zanon E, Bertomoro A, Girolami A. Do hemophilia A and von Willebrand disease protect against carotid atherosclerosis? A comparative study between coagulopathics and normal subjects by means of carotid echo-color Doppler scan. *Clin Appl Thromb Hemost* 1999; **5**: 232–5.

14 Bilora F, Boccioletti V, Zanon E, Petrobelli F, Girolami A. Hemophilia A, von Willebrand disease, and atherosclerosis of abdominal aorta and leg arteries: factor VIII and von Willebrand factor defects appear to protect abdominal aorta and leg arteries from atherosclerosis. *Clin Appl Thromb Hemost* 2001; **7**: 311–13.

15 Bilora F, Zanon E, Petrobelli F, Cavraro M, Prandoni P, Pagnan A, Girolami A. Does hemophilia protect against atherosclerosis? A case-control study. *Clin Appl Thromb Hemost* 2006; **12**: 193–8.

16 Ali YS, Rembold KE, Weaver B, Wills MB, Tatar S, Ayers CR, Rembold CM. Prediction of major adverse cardiovascular events by age-normalized carotid intimal medial thickness. *Atherosclerosis* 2006; **187**: 186–90.

17 Budoff MJ, Shaw LJ, Liu ST, Weinstein SR, Mosler TP, Tseng PH, Flores FR, Callister TQ, Raggi P, Berman DS. Long-term prognosis associated with coronary calcification: observations from a registry of 25 253 patients. *J Am Coll Cardiol* 2007; **49**: 1860–70.

18 Chambless LE, Heiss G, Folsom AR, Rosamond W, Szklo M, Sharrett AR, Clegg LX. Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) Study, 1987–1993. *Am J Epidemiol* 1997; **146**: 483–94.

19 Kondos GT, Hoff JA, Sevrukov A, Daviglus ML, Garside DB, Devries SS, Chomka EV, Liu K. Electron-beam tomography coronary artery calcium and cardiac events: a 37-month follow-up of 5635 initially asymptomatic low- to intermediate-risk adults. *Circulation* 2003; **107**: 2571–6.

20 Vliegenthart R, Oudkerk M, Hofman A, Oei HH, van Dijck W, van Rooij FJ, Witteman JC. Coronary calcification improves cardiovascular risk prediction in the elderly. *Circulation* 2005; **112**: 572–7.

21 Graham I, Atar D, Borch-Johnsen K, Boysen G, Burell G, Cifkova R, Dallongeville J, De Backer G, Ebrahim S, Gjelsvik B, Herrmann-Lingen C, Hoes A, Humphries S, Knapton M, Perk J, Priori SG, Pyorala K, Reiner Z, Ruilope L, Sans-Menendez S, *et al*. European guidelines on cardiovascular disease prevention in clinical practice: executive summary: Fourth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (Constituted by representatives of nine societies and by invited experts). *Eur Heart J* 2007; **28**: 2375–414.

22 Conroy RM, Pyorala K, Fitzgerald AP, Sans S, Menotti A, De Backer G, De Bacquer D, Ducimetiere P, Jousilahti P, Keil U, Njolstad I, Oganov RG, Thomsen T, Tunstall-Pedoe H, Tverdal A, Wedel H, Whincup P, Wilhelmsen L, Graham IM. Estimation of 10-year risk of fatal cardiovascular disease in Europe: the SCORE project. *Eur Heart J* 2003; **24**: 987–1003.

23 Stevens RJ, Kothari V, Adler AI, Stratton IM. The UKPDS risk engine: a model for the risk of coronary heart disease in Type II diabetes (UKPDS 56). *Clin Sci (Lond)* 2001; **101**: 671–9.

24 Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol* 1990; **15**: 827–32.

25 Groen JM, Greuter MJ, Vliegenthart R, Suess C, Schmidt B, Zijlstra F, Oudkerk M. Calcium scoring using 64-slice MDCT, dual source CT and EBT: a comparative phantom study. *Int J Cardiovasc Imaging* 2008; **24**: 547–56.

26 Folsom AR, Kronmal RA, Detrano RC, O'Leary DH, Bild DE, Bluemke DA, Budoff MJ, Liu K, Shea S, Szklo M, Tracy RP, Watson KE, Burke GL. Coronary artery calcification compared with carotid intima-media thickness in the prediction of cardiovascular disease incidence: the Multi-Ethnic Study of Atherosclerosis (MESA). *Arch Intern Med* 2008; **168**: 1333–9.

27 Greenland P, Bonow RO, Brundage BH, Budoff MJ, Eisenberg MJ, Grundy SM, Lauer MS, Post WS, Raggi P, Redberg RF, Rodgers GP, Shaw LJ, Taylor AJ, Weintraub WS, Harrington RA, Abrams J, Anderson JL, Bates ER, Grines CL, Hlatky MA, *et al*. ACCF/AHA 2007 clinical expert consensus document. *Circulation* 2007; **115**: 402–26.

28 Rumberger JA, Brundage BH, Rader DJ, Kondos G. Electron beam computed tomographic coronary calcium scanning: a review and guidelines for use in asymptomatic persons. *Mayo Clin Proc* 1999; **74**: 243–52.

29 Terpstra WF, May JF, Smit AJ, Graeff PA, Meyboom-de Jong B, Crijns HJ. Effects of amlodipine and lisinopril on intima-media thickness in previously untreated, elderly hypertensive patients (the ELVERA trial). *J Hypertens* 2004; **22**: 1309–16.

30 Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. *Circulation* 1997; **96**: 1432–7.

31 Baldassarre D, Nyyssonen K, Rauramaa R, De Faire U, Hamsten A, Smit AJ, Mannarino E, Humphries SE, Giral P, Grossi E, Veglia F, Paoletti R, Tremoli E. Cross-sectional analysis of baseline data to identify the major determinants of carotid intima-media thickness in a European population: the IMPROVE study. *Eur Heart J* 2010; **31**: 614–22.